Effect of uranium on growth and reproduction of the marine amphipod *Allorchestes compressa*

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Abstract

Experiments on growth of the marine amphipod *Allorchestes compressa* Dana were carried out over four weeks, and both growth and reproduction were studied over three generations, each of which was exposed to uranium for approximately 10 wk. At 0.1 mg l$^{-1}$ the uranium increased growth by 23%, as measured by the mean weight after 4 wk, and at 2 mg l$^{-1}$ growth was reduced by 28% compared with the control. *A. compressa* accumulated uranium from sea water with a concentration factor of 10. There was no effect of uranium on the survival of amphipods or their progeny in the multiple-generation experiment, but the numbers of males, the sex ratio, and the respiration rate (measured on males only) at 1 mg l$^{-1}$ were significantly lower than the control. *A. compressa* is shown to be a convenient species for the study of toxic effects on growth and reproduction using multiple-generation experiments.

Introduction

Uranium is being increasingly used in the nuclear industry and discharged to the aquatic environment (Chassard-Bouchaud, 1983; Chassard-Bouchaud et al., 1983), and it is also present in wastes from the phosphate industry (Oak Ridge National Laboratory, 1968) and coal-fired power stations (Valkovic, 1983). Australia has a large portion of the Western World's uranium reserves, and there is some risk of uranium pollution of sea water from accidents during the mining and milling operations or during export. Little has been reported on the effect of uranium on marine biota in general and none at all for Australian species. Concentrations of less than 0.1 mg l$^{-1}$ have been classified (National Academy of Sciences/National Academy of Engineering, 1973; Anonymous, 1979) as posing "minimal" risk to the marine environment, so this was used as a guide in selecting uranium concentrations for these experiments.

The marine amphipod *Allorchestes compressa* has many of the characteristics necessary for life-cycle studies over multiple generations in the laboratory (Ahsanullah and Brand, 1985). The purpose of the present work was to study the effect of uranium on growth and reproduction of *A. compressa* over three generations and to measure its ability to accumulate uranium. The chance of detecting the effects of uranium was maximised by initiating the experiments with juveniles, the stage reported for various crustaceans to be the most sensitive (Ahsanullah and Arnott, 1978).

Materials and methods

Test amphipods and acclimation

Stocks of laboratory-cultured *Allorchestes compressa* Dana and its food and habitat, the seagrass *Heterozostera tasmanica*, were obtained from the Marine Science Laboratory, Department of Conservation, Forests and Lands, Queenscliff, Victoria. Maintenance and acclimation procedures and the procurement of juveniles from females have been described elsewhere (Ahsanullah and Florence, 1984; Ahsanullah and Brand, 1985). All experiments were initiated with first-instar juveniles.

Test solutions and uranium measurements

Depleted uranyl nitrate [UO$_2$(NO$_3$)$_2$ $\cdot$ 6 H$_2$O, AR grade] in sea water was delivered to the experimental tanks from a dosing system similar to that described by Ahsanullah and Palmer (1980), but with a peristaltic pump in place of the flowmeters. The flow of toxicant was initiated 8 to 10 d before juveniles were introduced to precondition the tanks and seagrass.
The water was sampled weekly and analysed for uranium by two different methods: for the lower concentration (0.1 mg l⁻¹) by the direct fission-track method (Reimer, 1975) and, for the higher concentrations (1 and 2 mg l⁻¹), by the delayed neutron method (Wall, 1979).

Growth experiments

Four 10-liter Perspex tanks were dosed with sea water as control (~0.002 mg U l⁻¹), and nominally 0.1, 1.0 and 2.0 mg U l⁻¹. Each tank received 8 liters of sea water, 50 g of seagrass (wet weight) and about 100 one-day-old juveniles; the flow rate was adjusted to 1.6 liters per hour. Amphipods were sampled after 4 wk, dried at 60°C, and weighed. Experiments on growth (Experiments I, II and IV) were repeated three times. Four weeks was selected as the most convenient period because, after this time, differences between male and female growth began to appear. During the three-generation experiment (Experiment III), the amphipods were also sampled at four weeks for comparison with these experiments.

Accumulation

In the last four-week experiment (Experiment IV), enriched uranium (7% ²³⁵U) was used to increase the precision of measurement. Single composite samples of amphipods, seagrass and evaporated water from each uranium concentration were dried at 60°C, weighed and analysed by the delayed neutron method (Ellis and Ahsanullah, 1984).

Multiple-generation study

In six 60-liter glass tanks containing 40 liters of sea water and 200 g of seagrass, the flow rate was adjusted to 3.3 liters h⁻¹. Duplicate tanks were dosed with sea water (as control), 0.1 and 1.0 mg U l⁻¹. After preconditioning, about 200 (Table 2) one-day-old juveniles were introduced into each tank. At the end of about 10 wk, the amphipods and seagrass were removed from the tanks, and those amphipods recovered were sorted into pairs in amplexus, single males, single females and juveniles. They were counted, and females were examined for progeny. Because a large amount of labour would have been required at this stage to censor and change all tanks at the same time, the sampling period varied from 62 to 73 d (see Table 2). All females were exposed to anaesthetic and the number of young released were recorded. Female growth was estimated from length rather than from weight, on the assumption that the presence or absence of eggs would greatly increase the variation in weight.

Respiration rate was measured on male amphipods only, with a Gilson Differential Respirometer (Newell et al., 1972) using 15 ml reaction flasks at 20°C. Six males were used in each flask, with two control flasks containing sea water, and these were agitated at about one cycle per second. After 15 to 30 min incubation, measurements continued for 2 to 3 h and the results were expressed per unit dry body weight per unit time.

Exposure of the second and third generations of Allochrestes compressa was carried out in a similar way to the first, using freshly preconditioned seagrass and about 200 juveniles introduced directly from the previous generation. If sufficient juveniles were obtained from females within a tank, juveniles from that tank which appeared to be 1 d old were also included. In each generation, females that did not release any young were preserved in formalin and later dissected to count and record separately the numbers of undeveloped juveniles and eggs.

Data analyses

Two-way analysis of variance (ANOVA) and regression were used to analyse the growth data. Two-way ANOVA and analysis of covariance were used on the data from the multiple-generation experiment, where the main factors were the uranium concentration and the generation number; the duration of exposure was used as a covariate.

In the multiple-generation experiment there was some chance that a response in the second or third generation might be correlated with the response in the parent generation, particularly since the progeny were cultured in the same tank as the parents. The data were examined for such correlations before proceeding with other analyses.

Results

Effect of uranium on growth

The two-way ANOVA revealed no significant effect of uranium on growth of Allochrestes compressa (Table I).

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Mean individual weight (µg) at:</th>
<th>Control</th>
<th>0.1 mg U⁻¹</th>
<th>1.0 mg U⁻¹</th>
<th>2.0 mg U⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>648</td>
<td>nd</td>
<td>633</td>
<td>617</td>
<td></td>
</tr>
<tr>
<td>II*</td>
<td>592 ± 16</td>
<td>659 ± 25</td>
<td>581 ± 14</td>
<td>410 ± 17</td>
<td></td>
</tr>
<tr>
<td>III*</td>
<td>364</td>
<td>460</td>
<td>363</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>331</td>
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<td></td>
<td>179</td>
<td>243</td>
<td>320</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV*</td>
<td>235 ± 17</td>
<td>263 ± 12</td>
<td>111 ± 16</td>
<td>117 ± 13</td>
<td></td>
</tr>
</tbody>
</table>

* Error terms are ±1 SD of mean within a single tank for amphipods weighed in batches of five, using 12 replicates in Experiment II and 5 replicates in Experiment IV

* In Experiment III, data cover three generations, with two sets of tanks in each generation